Chapter I

Introduction to Carbohydrates and Polysaccharides

1.A. Carbohydrates

Carbohydrates are important biomolecules and they are the major source of energy in the living body, essential for the survival of plants and animals [1]. The general formula of carbohydrate is $C_x(H_2O)_y$. Water molecules present in the structure is not the water of crystallization and cannot be removed by heating. There is no free aldehyde or keto group in carbohydrates. They exist as hemiacetal or hemiketal formed by the condensation of the aldehyde or keto group with an alcoholic –OH group present in that molecule. It forms a major portion of our diet. Carbohydrates supply the required energy for metabolism occurring inside living beings. Polysaccharides like starch, glycogen are hydrolyzed enzymatically into glucose inside living cells. The produced glucose is then transported to all the cells via blood. After that oxidation of glucose into CO_2 and H_2O during enzyme catalyzed reactions yields energy.

$$\mathbf{C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 2880\ kJ.mol^{-1}}$$

1.A.1. Classification of carbohydrates

Carbohydrates are classified into three categories monosaccharide, oligosaccharide, and polysaccharide. Monosaccharides (ribose, glucose, fructose etc.) are chiral polyhydroxy aldehyde or ketone which cannot be hydrolysed to produce simpler carbohydrate. Oligosaccharides (sucrose, lactose, raffinose etc.) are composed of monosaccharide units (2-10) bound together by glycosidic linkages. Disaccharides are composed of two similar or different monosaccharides units. Polysaccharides (starch, cellulose, glycogen, etc.) are glycosidically linked several monosaccharide units.

Reducing sugars (glucose, maltose, lactose etc.) and non-reducing sugars (sucrose, starch etc.) are found in nature and classified on the basis of their reducing properties. Some sugars like glucose, sucrose possess sweet taste but the polymer of glucose like starch and cellulose are tasteless.

1.B. Polysaccharides – the major component of carbohydrates

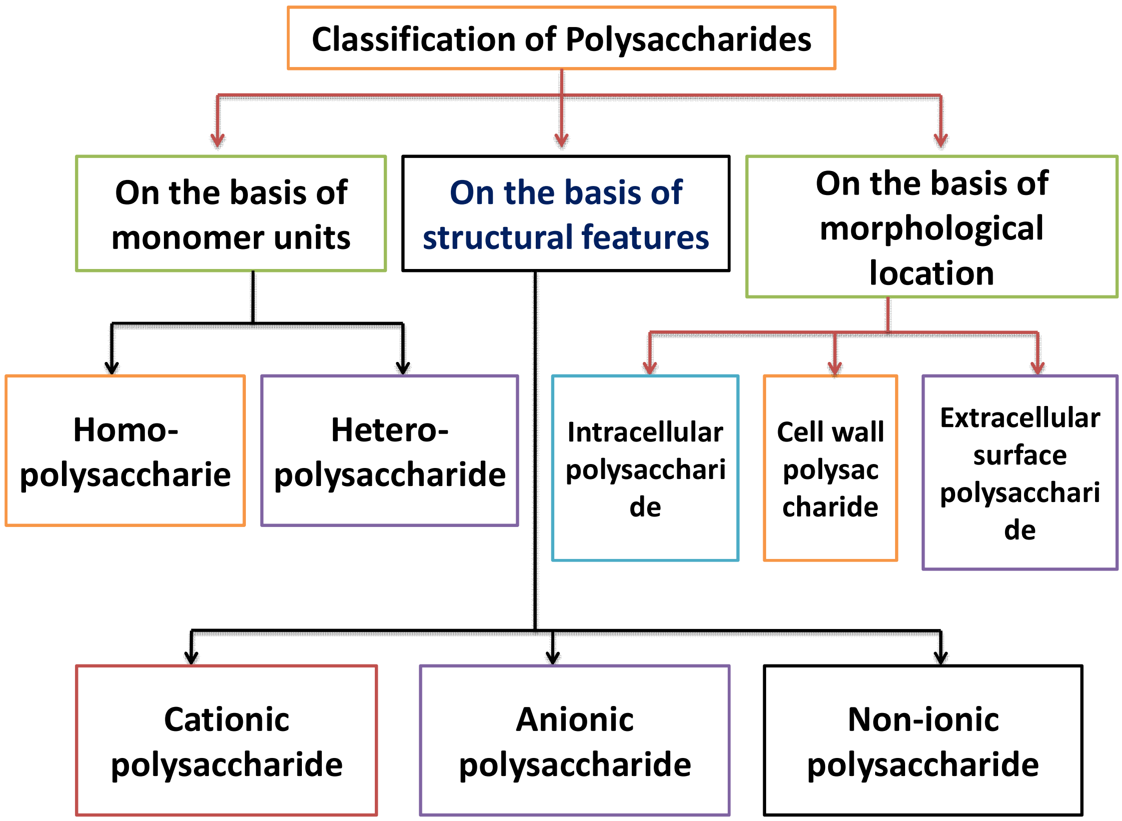
Carbohydrates which, on hydrolysis, produce several monosaccharide units are called polysaccharides. General formula of most of the polysaccharides is $(C_6H_{10}O_5)_n$. The earliest biopolymers [2] on Earth are polysaccharides which are formed by joining several monosaccharide units through glycosidic linkages. These are tasteless, amorphous, and insoluble in water. Polysaccharides exist abundant in the nature, including plants [3], mushrooms [4], bacterial cell wall [5], oil of palm fruit [6], and different vegetables [7,8] etc. Studies have demonstrated that polysaccharides not only play support and storage functions in living body, but also participate in the process of life activities.

1.B.1. Classification of polysaccharides

Classification of polysaccharides is shown in schematically. On the basis of the monosaccharide units, polysaccharides are classified into two categories namely homopolysaccharide and heteropolysaccharide. Homopolysaccharides constitute of only one type of monomer units e.g. starch, glycogen, cellulose etc. On the other hand polysaccharides composed of different type of monomer units are known as heteropolysaccharides e.g. glucomannan, galactomannan, arabinoxylan etc.

On the basis of structural features, polysaccharides are divided mainly into three categories specifically cationic polysaccharides (e.g. chitosan, cationic hydroxyethylcellulose etc.), anionic polysaccharides (e.g. pectin, hyaluronic acid, alginic acid, carboxymethyl-chitin etc.), and non-ionic polysaccharides (starch, dextrin, cellulose ethers etc.).

On the basis of morphological location polysaccharides are divided into three types specifically intracellular polysaccharides placed inside the cytoplasmic membrane of microorganisms, cell wall polysaccharide of living microbial cells, and extracellular surface polysaccharide situated outside the cell membrane of microbes.



❖ Schematic presentation of Classification of polysaccharides

1.B.2. Some common polysaccharides

➢ **Starch**

Starch is a polysaccharide formed only of D-glucose residues. It is amorphous granular solid and insoluble in cold water. Starch is a mixture of amylose (15-20%) and amylopectin (80-85%) [9]. Amylose is a linear polymer of α-D-glucose in which C-1 of one glucose unit is linked to C-4 of another by α-glycosidic linkage (**Fig. 1**). Amylopectin is a complex polymer containing of 10-200 branched glucose units. Starch is stored as a reserve food in tube, root, and seeds.

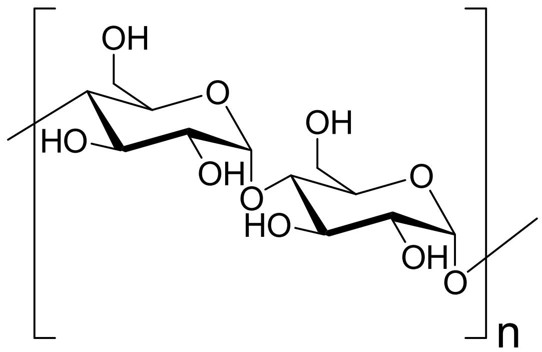


Figure 1: α-amylose, the linear polymer of α-D-glucose.

➢ **Glycogen**

Glycogen, a homopolysaccharide has the similar structure like as amylopectin with more extensive branching of glucose units. Animals use it for the short-term storage of food energy [10]. It is stored in liver and muscles of animals [11,12], and is called as 'animal starch'.

➢ Cellulose

Cellulose is a polysaccharide formed of only D-glucose residues. It is the major component of plant cell walls [13]. Cellulose is a linear polymer of serially arranged β-D-glucose units linked by β-(1→4)-glycosidic bonds (**Fig. 2**). Human intestine does not produce the enzyme cellulase which can hydrolyze cellulose into glucose. So, human beings cannot digest cellulose.

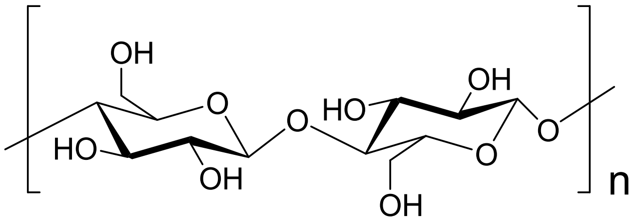


Figure 2: Cellulose, β-(1→4) linked glucose polymer.

➢ Chitosan

Chitin is composed of β-(1→4)-linked N-acetyl-D-glucosamine units. Chitosan (**Fig. 3)** is produced by the removal of N-acetyl groups from chitin. It is found from the external skeleton of shellfish, as well as from lobster, crab, and shrimp. Chitosan is used in pharmaceutical industry as filler in tablets as a carrier in controlled drug delivery and also used in the treatment of patients of kidney failure.

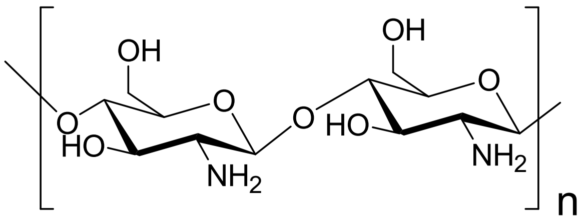


Figure 3: Chitosan

➢ Pectin

Pectin is heterogeneous polysaccharides. Pectin contain α-(1→4)-linked galacturonic acid or its ester in the backbone. Pectin (**Fig. 4**) plays an important role in human diet.

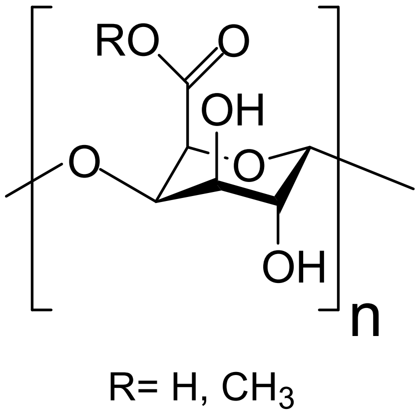


Figure 4: Pectin: α-(1→4)-linked galacturonic acid or its ester in the backbone.

➢ Heparin

Heparin is an acidic mammalian polysaccharide, composed of sulfated (1→4)-linked hexosamine and uronic acid residues (**Fig. 5**). It has a multiple biological utility e.g. blood anticoagulation, cell migration, mitogenesis etc.

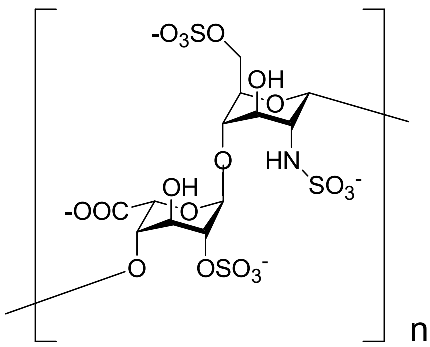


Figure 5: Heparin, sulfated (1→4)-linked hexosamine and uronic acid.

➢ Hyaluronic acid

Hyaluronic acid is a high molecular weight polysaccharide with a linear structure (**Fig. 6**). It is composed of disaccharide repeating units of glucuronic acid and N-acetyl glucosamine moieties. Due to presence of some biocompatibility and rheological properties [14] hyaluronic acid has a good number of medical applications.

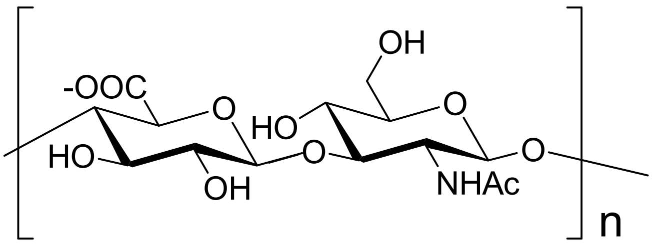


Figure 6: Hyaluronic acid.

1.C. Mushrooms

The term '**mushroom**' is used as fruit body of higher fungi, grown mostly above ground. Mushrooms have a long history of use as food and traditional medicine [15]. Generally fruiting body of mushroom consists of a cap or pileus and a stalk or stipe but others have additional structures like scale, a cup or volva, gills, tubes, pores, ring etc. Mushroom species can be classified into three categories. Mycorrhizal or symbiotic species form a close, mutually favourable relationship with their host vascular plant, usually a tree. Saprotrophic species or saprophytes derive their nutrients from dead organic material. Some of these species are the basis for cultivated mushroom production, while mycorrhizal species have not yet been successfully cultivated. The third group of parasitic species lives on other species in a non-symbiotic relationship.

A number of species, e.g. the genus Agaricus, possess high levels of several trace elements, mainly cadmium and mercury contents may cause a risk [16]. Eating of those species, as well of mushrooms collected from polluted areas, should thus be restricted.

1.C.1. Chemical composition and nutritional value of edible mushrooms

Mushrooms are consumed as delicious food, particularly for their definite aroma and texture. Generally, mushrooms are low in energy and high in dietary fibre [17]. Wild edible mushrooms possess high nutritional and potential economic value. Many species with medicinal value are widely used in traditional medicine for a broad range of diseases [18]. It has been well established that cultivated mushrooms contain reasonable amounts of proteins, carbohydrates, minerals, and vitamins. Nutritionally, they rank between high grade vegetables and low grade meat [19]. Although mushrooms cannot directly substitute for meat, fish, or eggs, they can serve as a good substitute in preventing protein malnutrition [19]. Mushrooms are rich in proteins and certain essential amino acids [20] and dietary fiber which are valuable for human nutrition [21]. Mushrooms are rich and balanced sources of potassium, sodium, magnesium, calcium, iron, zinc, copper, and phosphorous. They are high in K and low in Na, so they are ideal foods for those want to reduce sodium intake for health reasons [22].

1.C.2. A wild edible mushroom: *Termitomyces clypeatus*

Termitomyces clypeatus, belongs to the family lyophyllaceae is a wild edible mushroom. It is brownish in colour, with white margin having reflexed roll. No colour change occurs on bruising or handling. It habitats on grassland and also found in tropical moist deciduous areas. This mushroom is comparatively prone to insects, hence, it is preferred to be plucked at immature stage. The mushroom was collected by Dr. Krishnendu Acharya, Professor, Department of Botany, University of Calcutta, West Bengal. A reference specimen was deposited at the Calcutta University herbarium (Accession no: CUH AM351).

Figure 7: Photograph of the fruit bodies of an edible mushroom, *Termitomyces clypeatus*.

1.C.3. An edible truffle *Tuber rufum* (Pico) var.: ectomycorrhizal mushroom

Tuber rufum (Pico) var., an ectomycorrhizal [23] fungus grows in association with moist deciduous trees and conifers during the late autumn and winter. It belongs to the group of edible mushroom known as truffle. The mutuality association of plant roots and fungi is beneficial to both organisms, and they live together through symbiotic mechanism. Trained pigs and dogs through their ability to detect and recognize the volatile aromatic chemicals produced by the truffles determine the underground locations of the truffles. The locations of truffles can be detected also by observing those locations where the flies (genus *Suillia*) fly; they lay eggs on the ground above truffles that, in turn, provide food for the larvae [24]. Truffles are one kind of precious food mushrooms and widely used in Asia and Europe for many years [25]. The popularity of truffles is believed to be due to their nutritional value and delicious taste. With hundreds of truffle species, not all of them are edible but those of genus *Tuber* serve as a food source [26].

Figure 8: Photograph of the fruit bodies of an ectomycorrhizal edible mushroom, *Tuber rufum* (Pico) var.

1.C.4. A wild edible mushroom: *Lentinus sajor-caju*

Lentinus sajor-caju is a saprophytic, wild edible mushroom belongs to the family polyporaceae. It grows abundantly on dead and fallen branches and trunks. This fungus is one of the wood decaying fungi. The colour of this mushroom is cream to brown. It is funnel shaped. The fruiting body of this mushroom is very tough. The mushroom was collected by Dr. Krishnendu Acharya, Professor, Department of Botany, University of Calcutta, West Bengal. A reference specimen was deposited at the Calcutta University herbarium (Accession no: CUH AM352).

Figure 9: Photograph of the fruit bodies of wild edible mushroom *Lentinus sajor-caju*

1.C.5. Mushroom polysaccharides

Mushrooms consist of biologically active polysaccharide present in fruit bodies. The polysaccharides are mainly β-glucans that are present in the cell walls [27]. Fungal β-glucans are well-known for their immune-modulating and tumour growth-inhibiting activities [28,29]. It has been suggested that the polysaccharides present in the mushrooms exert these effects. Some of the more effective compounds in mushrooms are (1→6)- and (1→3)-β-D-glucan which have been reported to inhibit tumour growth by stimulating the immune system through activation of macrophages, T helper cell and subsequent effects on natural killer cells (NK-cell) to produce cytokines [30]. There are also some reports on antitumor activity of heteroglycan polysaccharides [31]. Such mushroom polysaccharides are being evaluated as adjuvant cancer therapy compounds along with conventional cancer treatments [32]. If edible mushrooms are present in our normal diet, effects of mushrooms on (intestinal) immune responses are of potential interest for optimizing the functioning of our immune system.

1.D. Biological activities

A wide range of biological activities of various polysaccharides with different chemical structures from mushrooms have been investigated. Lymphocytes are important immunological cells and play a significant role in acquired immune system. Glutathione is present in the oxidized form (GSSG), which is readily converted to the reduced form (GSH) by the enzyme glutathione reductase. Malondialdehyde (MDA) and glutathione dependent enzyme levels determine the lipid and protein damage and the antioxidant status in lymphocytes respectively [33]. Reactive oxygen species (ROS) are formed after the cell goes through a normal metabolism of oxygen. These have very important roles in cell signaling and homeostasis. ROS, when in excess, inflict damage on macromolecules and ultimately lead to apoptosis or necrosis [34,35]. ROS generation may increase due to various environmental stresses like UV or heat exposure, which may result in significant damage to various cell structures. The magnitude of oxidative stress may determine the mode of cell death. A number of cellular antioxidant systems help to keep ROS in check. For instance, glutathione is believed to scavenge reactive oxygen species directly or act as a substrate for other antioxidative or repair enzymes. Glutathione peroxidase catalyzes the reduction of hydroperoxides, with glutathione (GSH) being oxidized to glutathione disulfide (GSSG). The later is converted to glutathione by glutathione reductase in the presence of NADPH. They also provide a link to adaptive immune system via recruitment of lymphocytes. The effects of oligosaccharides and polysaccharides on ROS production by mammalian cells can be correlated predicatively with overproduction [36].

Cell viability refers to the number of healthy cells in blood sample and it determines the amount of cells that are living or dead based on total cell sample. A viability assay determines the ability of organs, cells or tissues to maintain or recover viability. Addition of polysaccharides like soyabean meal polysaccharide in culture medium can significantly decrease the death of cells [37].

Among various factors, Nitric oxide (NO) is one of the important endogenous metabolites that enhances the generation of ROS. It has been considered that NO also plays a vital role in diverse physiological functions in living systems. NO has several effects on lymphocyte function. It can inhibit apoptosis. It serves as a facilitator of the immune response, promoting survival of lymphocyte populations. But NO has also been shown to inhibit lymphocyte proliferation and promote apoptosis and thus effectively prevent amplification of the immune response [38]. In spite of its potential role, in a series of studies, NO proved to be capable of regulating the multiple plant responses towards a variety of biotic and abiotic stresses and alleviating some consequences provoked by oxidative stresses [39]. As the cell wall of mushroom consists of a large part of the polysaccharide β-glucan, it has been suggested that these polysaccharides increased NO production [40].

1.E. Antioxidant activities

Free radicals, which are generated in several biochemical reactions in the body, have been implicated as mediators of many diseases, including cancer, rheumatoid arthritis, cardiovascular disease, atherosclerosis and heart diseases [41-43]. Reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radical are the byproducts of normal metabolism and attack certain biological molecules, leading to destabilization and disintegration of cell membranes and many age-related diseases [44]. They are also associated with oxidative damage to DNA, proteins and other macromolecules accumulates with age and has been postulated to be a major type of endogenous damage leading to aging [45].

Antioxidant supplements or foods containing high concentrations of antioxidants may help to reduce oxidative damage. Antioxidant compounds can scavenge free radicals and increase shelf life by retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food products during processing and storage. Synthetic antioxidants have been used in stabilization of foods. Synthetic compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), and tert-butylated hydroxyquinone (TBHQ) which are effective in their role as antioxidants, are commercially available and currently used in industrial processes. They are applied in fat and oily foods to prevent oxidative deterioration [46]. Recently, it has been established that tumour formation is promoted by BHA and BHT [47]. However, since suspected actions as promoters of carcinogenesis and other side effects have been reported, their use in food, cosmetic and pharmaceutical products has been decreasing [48].

Thus, there has been an upsurge of importance in naturally-occurring antioxidants from food, because it is believed that they can protect the human body from the attack of free radicals and retard the progress of many chronic diseases, as well as retarding the lipid oxidative rancidity in foods [49]. So, it is necessary for identifying alternative natural antioxidants, especially of plant origin, has notably increased in recent years. Mushrooms contain various polyphenolic compounds recognized as an excellent antioxidant due to their ability to scavenge free radicals by single-electron transfer [50]. Polysaccharides are potentially useful biologically active ingredients for pharmaceutical use, such as for immune regulation, for anti-radiation, anti-blood coagulation, anti-cancer, anti-HIV and hypoglycemic activity [51,52]. The objective of the present study was to evaluate and compare antioxidant properties and biological effects of hot water extracted polysaccharides from the edible wild mushroom species.

1.F. Conclusions

The activity of mushroom polysaccharide may depend on their structural properties, such as polymer length, degree of branching, tertiary structure, and molecular weight [53]. The aim of this study was to investigate the structural characterization as well as antioxidant activities and biological effects of polysaccharides isolated from different mushroom species.